STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory sample requirements (see CP item 33 & 34) _____

STANDARD PLATE AND COLIFORM METHODS

DILUTING SAMPLES

2. Work Area _____

   a. Level plating bench not in direct sunlight _____

   b. Sanitized immediately before start of plating _____

3. Selecting Dilutions _____

   a. Standard Plate Count _____

      1. Plate two decimal dilutions per sample _____

      2. Select dilutions to yield one plate with 25-250 colonies _____

         a. Raw milk is normally diluted to 1:100 and 1:1000 _____

         b. Finished products are normally diluted to 1:10 and 1:100 _____

         c. The above are general guidelines and may have to be adjusted on a case by case basis (dilutions below 1:10 not required) _____

   b. Coliform Counts _____

      1. For milk samples, 1 mL direct and/or decimal dilutions _____

      2. For all other products, distribute 10 mL of a 1:10 dilution among three plates, generally high fat and viscous products _____

4. Identifying Plates _____

   a. Label each plate with sample identification and dilution _____

   b. Arrange plates in order before preparation of dilutions _____

5. Sample Agitation _____

   a. When appropriate, wipe top of unopened containers with sterile, ethyl alcohol-saturated cloth _____

b. Before removal of any portion, thoroughly mix contents of each container _____

1. Shake raw and processed sample containers (approx ¾ full) 25 times in 7 sec with l ft movement _____

2. Invert filled retail container 25 times, each inversion a complete down and up motion _____

c. Remove test portion within 3 min of sample agitation _____

6. Sample Measurement, pipets _____

a. Use separate sterile pipets for the initial transfers from each container _____

1. Pipets in pipet container adjusted without touching the pipets _____

b. Pipet tip not dragged over exposed exterior of pipets in container _____

c. Pipet not dragged across lip or neck of sample container _____

d. Pipet not inserted more than 2.5 cm (1") below sample surface (foam avoided if possible) _____

e. Draw test portion above pipet graduation mark and remove pipet from liquid _____

1. Pipet aid used, mouth pipetting not permitted (_____) _____

f. Adjust test volume to mark with lower side of pipet in contact with inside of sample container (above the sample surface) _____

g. Drainage complete, excess liquid not adhering to pipet _____

h. Release test portion to petri dish (tip in contact with plate, 45° angle) or dilution blank (with lower side of pipet in contact with neck of dilution blank, or dry area above buffer where appropriate) with column drain of 2-4 sec _____

i. Blow out last drop of undiluted sample from pipet using pipet aid _____

1. Blow out away from main part of sample in plate, do not make bubbles _____

j. Pipets discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized _____

7. Sample Measurement, pipettors (_____) _____

a. Each day before use, vigorously depress plunger 10x to redistribute lubrication and assure smooth operation (mechanical pipettors) _____

b. Before each use examine pipettor to assure that no liquid is expelled from the pipettor nose-cone (contaminated), if fouling is detected do not use until cleaned as per manufacturer recommendation _____

c. Use separate sterile tip for the initial transfers from each container _____

d. Depress plunger to first stop (mechanical pipettors) _____

e. Tip/barrel not dragged across lip or neck of sample container, and pipettor barrel not allowed within sample container _____

f. Tip not inserted more than 1 cm below sample surface (foam avoided if possible) _____

g. With pipettor vertical **slowly** and completely release plunger (for electronic pipettors follow manufacturer instructions) _____

h. Remove tip from sample and depress plunger completely, re-insert tip into sample and repeat steps f and g, and then remove tip from liquid _____

i. Touch tip off to inside of sample container above the sample surface, excess liquid not adhering to tip (do not lay pipettor down once sample is drawn up, use vertical rack if necessary) _____

j. Release test portion to petri dish (tip in contact with plate) by slowly depressing plunger to first stop allowing about 1 or 2 seconds for complete drainage _____

k. Move tip to a dry spot on plate _____

1. If pipettor only has one (1) stop touch off _____

2. If pipettor has two (2) stops, depress plunger to second stop and touch off _____

l. Or, dispense test portion to dilution blank (tip in contact neck of dilution blank, or dry area above buffer where appropriate) by slowly depressing plunger to first stop _____

m. If pipettor has two (2) stops, depress plunger to second stop _____

n. Tips discarded into disinfectant, biohazard bags or containers to be sterilized _____

8. Dilution Agitation _____

a. Before removal of any portion, shake each dilution bottle 25 times in 7 sec with a 1 ft movement _____

b. Optionally, use approved mechanical shaker for 15 sec _____

c. Remove test portion within 3 min of dilution agitation _____

9. Dilution Measurement, pipets _____

a. Use separate sterile pipets for the initial transfers from each container _____

1. Pipets in pipet container adjusted without touching the pipets _____

b. Pipet tip not dragged over exposed exterior of pipets in container _____

c. Pipet not dragged across lip or neck of dilution blank _____

d. Pipet not inserted more than 2.5 cm (1") below dilution surface _____

e. Draw dilution portion above pipet graduation mark and remove pipet from liquid _____

1. Pipet aid used, mouth pipetting not permitted (_____) _____

f. Adjust dilution volume to mark with lower side of pipet in contact with inside of dilution blank neck _____

g. Drainage complete, excess liquid not adhering to pipet _____

h. Gently lift cover of petri dish just high enough to insert pipet _____

i. Hold pipet at 45° angle to dish with tip touching dish (or dilution blank neck) _____

j. Release dilution portion to dish (or dilution blank) with tip in contact with the bottom of the dish (or dilution blank neck, or dry area above buffer where appropriate) with column drain of 2-4 sec _____

k. Touch pipet tip once against dry spot on dish bottom (or dilution blank neck) _____

l. When measuring 0.1 mL, do not re-touch dry area _____

m. Pipets discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized _____

10. Dilution Measurement, pipettors (_____) _____

    a. Use separate sterile tip for the initial transfers from each container _____

    b. Depress plunger to first stop (mechanical pipettors) _____

    c. Tip/barrel not dragged across lip or neck of dilution blank, and pipettor barrel not allowed within dilution blank _____

    d. Tip not inserted more than 1 cm below dilution surface _____

    e. With pipettor vertical **slowly** and completely release plunger (for electronic pipettors follow manufacturer instructions) _____

    f. Remove tip from dilution and depress plunger completely, re-insert tip into dilution and repeat steps d and e, and then remove tip from liquid _____

    g. Touch tip off to inside of dilution blank neck or dry area above buffer where appropriate, excess liquid not adhering to tip _____

    h. Gently lift cover of petri dish just high enough to insert tip _____

    i. Hold pipettor nearly vertical to dish with tip touching dish _____

    j. Release test portion to petri dish (tip in contact with plate) by slowly depressing plunger to first stop _____

    k. Move tip to a dry spot on plate _____

        1. If pipettor only has one (1) stop touch off _____

        2. If pipettor has two (2) stops, depress plunger to second stop and touch off _____

    l. Tips discarded into disinfectant, biohazard bags/containers or into spent dilution blanks to be sterilized _____

11. Samples Other than Milk _____

    a. Weigh 11g aseptically into dilution blank _____

    b. Use dilution blanks heated to 40-45C _____

12. Dry Milk Samples _____

    a. Weigh 11g aseptically into dilution blank heated to 40-45C _____

        1. Use standard dilution blank _____

2. Or, 2.0% sodium citrate blank (pH<8.0) for relatively insoluble sample (not to be used with Petrifilm) _____

b. Wet sample completely with gentle agitation (invert) _____

c. Let soak 2 min, then shake 25 times in 7 sec with l ft movement, use within 3 minutes of agitation _____

PLATING

13. Plating _____

a. Melt agar quickly in boiling water, flowing steam not under pressure, or microwave oven (use extreme caution when microwaving) _____

b. Avoid prolonged exposure to high temperatures during and after melting, establish lab protocol _____

c. Do not melt more than will be used within 3 hours _____

d. Do not melt agar more than once _____

e. Promptly cool melted agar to 45±1C _____

1. Record temperature with other control information _____

f. Temperature control used for each test medium type _____

1. Contains medium identical to type being used _____

2. In container identical to that being used _____

3. Undergoes <u>same</u> heat treatment and cooling as test medium _____

g. Select number of samples in any series so that all will be plated within 20 min (pref Ω#10) after diluting first sample _____

h. After depositing test portions, promptly pour 10-12 mL medium into each plate of series, or 15-20 mL for > 1 mL portion/plate or where agar weight loss is a problem that can not be corrected by other actions (documentation must be kept to indicate that this is a routine practice) _____

i. Lift cover of petri dish just high enough to pour medium _____

j. As each plate is poured thoroughly and evenly mix medium and test portion in petri dish _____

1. Multiple plates may be poured and mixed, however, plates may not be stacked prior to mixing _____

k. Allow to solidify within 10 min on level surface _____

l. For dry milk sample, overlay plate with 3-5 mL PCA _____

m. For coliform count, overlay plate with 3-4 mL VRB _____

n. Invert and incubate within 10 min of medium solidification _____

CONTROLS

14. Controls _____

a. Check sterility of dilution blanks, medium, petri dishes, and pipets used for each group of samples (AM and PM) _____

b. Expose a poured plate with cover completely removed or pre-hydrated Petrifilm Aerobic Count (PAC) film (both wet surfaces completely exposed) to air for 15 min during plating, AM and PM _____

1. The air control plate must be the first plate poured immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side) _____

2. If count > 15, take and note corrective actions _____

3. For PAC films see item 45b7 _____

c. Records maintained _____

d. Include information on bench, work sheet or report sheet(s) _____

INCUBATION

15. Incubation _____

a. Incubate SPC plates at 32±1C for 48±3 hours (dry milk for 72±3 hours) and incubate coliform plates at 32±1C for 24±2 hours _____

b. Stack plates no more than 6 high _____

c. Arrange stacks so each is at least 1" from adjacent stacks and from incubator surfaces _____

d. Place stacks directly over each other on successive shelves _____

COUNTING COLONIES

16. Counting Aids _____

a. Count colonies with aid of magnification under uniform and properly controlled artificial illumination with a hand tally _____

17. Recording Standard Plate Count _____

a. After incubating plates, promptly count all colonies on selected plates _____

b. Where impossible to count at once, store plates at 0-4.4C for not longer than 24 hr (avoid as a routine practice) _____

c. Record dilutions used and number of colonies on each plate counted _____

d. Record results of sterility and control tests _____

e. When possible, select spreader free plates with 25-250 colonies and count all colonies including those of pinpoint size _____

1. Use higher magnification if necessary to distinguish colonies from foreign matter _____

2. Examine edge of petri plates for colonies _____

f. If consecutive plates yield 25-250 colonies, count all colonies on plate(s) from both dilutions _____

g. Spreaders _____

1. Count colonies on representative portion only when colonies are well distributed and area covered or repressed does not exceed 25% of plate _____

2. Do not count if repressed growth area > 25% of plate area _____

3. When spreaders must be counted, count each as a single colony _____

4. Count chains/spreaders from separate sources as separate colonies _____

5. If 5% of plates are more than ¼ covered by spreaders, take immediate steps to eliminate and resolve problem _____

h. If there is no 25-250 colony plate, use plate having nearest to 250 colonies _____

i. If plates from all dilutions exceed 250 colonies, estimate counts as follows _____

1. Count colonies in portions representative of distribution and estimate total _____

2. Where there are < 10 colonies/sq cm, count colonies in 12 squares, selecting 6 consecutive squares horizontally across the plate and six consecutive squares at right angles _____

3. When there are 10 or more colonies/sq cm, count 4 representative squares _____

4. Multiply average number colonies/sq cm by area of plate in sq cm _____

j. If plates yield < 25 colonies each, record actual number in lowest dilution _____

k. If all plates from a sample show no colonies, record count as 0 _____

18. Coliform Count _____

a. After incubating plates, promptly count colonies _____

b. Where impossible to count at once, store plates at 0-4.4C for not longer than 24 hr (avoid as a routine practice) _____

c. Dark red colonies measuring 0.5 mm or more in diameter on agar plates are considered coliforms in plates containing Ω#154 colonies _____

d. On crowded plates, coliform colonies may be atypical; count and confirm presence of lactose fermentors _____

e. Confirmation of colonies _____

1. Pick 10% up to 10 representative colonies per plate with relative percentages of each colony type and inoculate into brilliant green lactose bile broth; incubate 24-48 hr at 32±1C as appropriate _____

2. Presence of any gas in a BGB tube constitutes a confirmed test _____

3. Record the number of picked colonies and the number of colonies that produced gas (if necessary calculate % confirmed and multiply by total number of colonies) _____

f. If no colonies appear on plate(s), record count as 0 _____

g. If there are 1-154 colonies on a plate, record number counted _____

h. If > 154 colonies develop in highest dilution plate, record number as > 150 _____

i. When multiple plates of a dilution are used, sum counts of plates _____

19. Personal Errors _____

a. Avoid inaccurate counting due to carelessness, fatigue, or impaired vision _____

b. Discover cause and correct if unable to duplicate your own counts on the same plate _____

c. Perform monthly counting _____

1. If 3 or more analysts use the RpSm method, see current SMEDP, records maintained _____

2. If less than three analysts, comparative counts agree Ω8% for the same analyst and Ω10% between two analysts, records maintained _____

REPORTS

20. Computing and Reporting Counts _____

a. Multiply number of colonies (or estimated number if necessary) by the reciprocal of the dilution _____

b. If consecutive dilutions yield 25-250 colonies, compute count using formula below (see current SMEDP) _____

$$N = OC/[(1 \times n_1) + (0.1 \times n_2)]d$$

Where, N = number of colonies per milliliter or gram
OC = sum of all colonies on all plates counted
n_1 = number of plates in lower dilution counted
n_2 = number of plates in next highest dilution counted
d = dilution from which the first counts were obtained

Example: 1:100 = 244 colonies 1:1,000 = 28 colonies

$N = (244 + 28)/ [(1 \times 1) + (0.1 \times 1)]0.01$
$= 272/[1.1]0.01$
$= 272/0.011$
$= 24,727$ [25,000 (reported)]

Note: In the NCIMS Program the denominator will always be 0.11 for 1:10 dilutions and 0.011 for 1:100 dilutions

c. Report SPC and coliform counts only if inhibitors are <u>not</u> detected _____

d. Report computed count as Standard Plate Count/mL or /g (SPC/mL or SPC/g) when taken from plate(s) in the 25-250 range _____

e. Report count as Coliform Count (confirmed)/mL or /g when taken from plate(s) in the l-l54 range _____

f. If no colonies appear on SPC plates, report as < 25 times the reciprocal of the dilution and report as estimated _____

g. If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as estimated _____

h. Report SPC plate counts of 0 to 24 as < 25 times the reciprocal of the dilution and report as estimated _____

i. When colonies on SPC plates exceed 100/sq cm, compute count by multiplying 100 x dilution factor x area of plate in sq cm and report as > computed count estimated _____

j. Computed counts from SPC plates outside the 25-250 range are reported as Estimated SPC (ESPC) _____

k. Counts from coliform plates > 154 are reported as > 150 Estimated Coliform Count (ECC) _____

l. If for any reason, an entire plate is not counted, the computed count is reported as Estimated _____

m. Report only first two left-hand digits _____

1. If the third digit is 5 round the second number using the following rules _____

a. When the second digit is odd round up (odd up, 235 to 240) _____

b. When the second digit is even round down (even down, 225 to 220) _____

n. If all plates from a sample have excessive spreader growth, report as spreaders (SPR), or are known to contain inhibitor(s) report as growth inhibitors (GI) _____

o. If a laboratory accident renders a plate uncountable, report as laboratory accident (LA) _____

PLATE LOOP COUNT METHOD

APPARATUS

21. Loop 0.001 mL _____

a. True circle, welded I.D. 1.45±0.06 mm, calibrated to contain 0.001 mL, made of appropriate wire _____

b. Loop fits over a No. 54 but not a No. 53 twist drill bits (lab must have set), checked monthly, records maintained _____

c. Modified by making a 30° bend 3-4 mm from loop, compare to template before use _____

d. Opposite end of wire kinked in several places _____

22. Hypodermic Needle, Luer-Lok _____

a. 13 gauge (sawed off 24-36 mm from the point where the barrel enters the hub) _____

b. Kinked end of loop wire shank inserted into needle until bend is 12-14 mm from end of barrel, compare to template before use _____

23. Cornwall Continuous Pipetting Outfit _____

a. Consisting of metal holder, Cornwall Luer-Lok syringe and filling outfit _____

b. Syringe, 2 mL capacity, adjusted to deliver 1.0 mL _____

1. Calibrated by checking ten 1 mL discharges (10 mL) using a 10 mL Class A graduate cylinder each day of use, records maintained _____

c. With Luer-Lok of needle attached to Luer-Lok fitting of syringe _____

PREPARATION

24. Heat Treatment of Pipetting Equipment _____

a. Sterilize assembled outfit wrapped in kraft paper or in a closed container by autoclaving at 120±1C for 15 min _____

25. Assembly of Complete Apparatus for Use _____

a. Place end of rubber supply tube (attached to syringe) in sterile dilution buffer blank and depress syringe plunger several times to pump buffer into syringe _____

b. Briefly flame loop and allow to cool 15 sec _____

c. Discharge several 1 mL portions to waste, then discharge 1 mL portion of buffer into instrument control plate _____

PROCEDURE

26. Comparative Test with SPC _____

a. Comparisons done by each analyst performing test _____

1. Comparison is valid only if done using similar plate count methods, i.e. SPC agar with pipets (or pipettors) to SPC agar with the PLC device or Petrifilm with pipets (or pipettors) to Petrifilm with the PLC device. Mixing methods is not permissible _____

2. Results must be shown to be acceptable prior to official use of test in laboratory _____

b. Copy of comparison and results in QC record (or easily accessible file in laboratory) _____

27. Identifying Plates (as item 4) _____

28. Sample Agitation (as item 5) _____

29. Inoculating Plates _____

a. Dip loop into each sample (avoiding foam) to bend in shank and withdraw vertically from surface three times in 3 sec with uniform movement of 2.5 cm _____

b. Raise cover of petri dish (just high enough to insert loop), insert loop and depress plunger causing sterile dilution buffer to flow across charged loop washing measured 0.001 mL of sample into dish _____

c. Do not depress plunger so rapidly that buffer fails to flow across loop _____

30. Plating _____

a. As described in item 13 or 44 _____

b. Pour plates with 12-15 mL agar _____

CONTROLS

31. Controls _____

a. See item 14 _____

b. Initial rinse control, see item 25c and 29c _____

c. Determine if loop is free rinsing by preparing a rinse control plate after every 20 samples plated _____

d. After all samples have been run discharge a final rinse to a control plate _____

INCUBATION

32. Incubation (see item 15) _____

a. 48±3 hr at 32±1C _____

COUNTING COLONIES

33. Counting Aids (see item 16 or 47b) _____

34. Recording Plate Loop Counts (see item 17 or 48) _____

35. Personal Errors (see item 19 or 49) _____

REPORTS

36. Reporting Counts _____

    a. See item 20 or 50 _____

    b. If 0 to 24 colonies on plate report as < 25,000 Estimated Plate Loop Count/mL (EPLC/mL) or if Petrifilm used, EPPLC/mL _____

    c. If count is between 25 and 250, report count as PLC/mL or PPLC/mL _____

    d. If colony count is > 250, report as EPLC/mL or EPPLC/mL _____

    e. When colonies exceed 100/sq cm, compute count by multiplying 100 x dilution factor x area of plate in sq cm and report as > computed count estimated _____

PETRIFILM AEROBIC COUNT METHOD

APPARATUS

37. Petrifilm Aerobic Count (PAC) Films _____

38. Plastic Spreader _____

    a. Provided with Petrifilm films, concave (ridge) side used _____

PROCEDURE

39. Identifying Films (as item 4) _____

40. Sample Agitation (as item 5) _____

41. Sample Measurement (as item 6&7) _____

42. Dilution Agitation (as item 8) _____

43. Dilution Measurement (as item 9&10) _____

44. Procedure _____

    a. Place the film on a level surface _____

    b. Lift the top film and deposit 1 mL of sample or dilution onto the center of the base film, touching off the last drop _____

1. Deposit samples with pipet (since only 1 mL samples can be used; 10 fold dilution will have to be made) _____

2. Or, deposit samples with pipettor (capable of making a 1:10 dilution in the tip) _____

3. Or, deposit sample with PLC apparatus (item 29) _____

c. Carefully **drop** the top film onto the inoculum _____

d. Place the plastic spreader with the ridge side down (item 38) on the top film over the sample and press down gently on the center of the spreader to distribute inoculum to the circular ridge of the spreader _____

e. Leave film undisturbed for 1 min for gel solidification _____

f. Incubate within 10 min of solidification _____

45. Controls _____

a. See item 14 above except for air plates _____

b. Air plates _____

1. Inoculate PAC film with dilution buffer (1 mL) _____

2. Drop film down onto the dilution buffer and spread as described in item 44d above _____

3. Leave film undisturbed for 1 minute for solidification of gel _____

4. The film must be the first one prepared immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side) _____

5. Roll top film back and away from bottom film and expose film for 15 min _____

6. After the 15 min roll top film back down and incubate with other films as usual _____

7. Incubated, exposed films should contain Ò 10 colonies, if count > 10, take and note corrective actions _____

INCUBATION

46. Incubation _____

a. Place films in horizontal position, clear side-up _____

b. Stack films no more than 20 high _____

c. Incubate 48±3 hr at 32±1C _____

COUNTING COLONIES

47. Counting PAC Films _____

    a. See item 16, or _____

    b. Optionally, count using an approved Petrifilm reader _____

        1. Refer to manufacturer's instructions for set-up and operation information _____

        2. 3M Petrifilm Information Management System (PIMS) _____

            a. Store control cards in a clean, dry and enclosed container _____

            b. Scan and record control card result prior to the start of and at the end of each operation period _____

            c. Scan and record control card result hourly with continuous operation _____

            d. Control card result must fall in the 92 to 108 range, if outside of this range an alarm will sound to alert the operator of a failure _____

                1. If alarm sounds, inspect card for defects, if defect(s) are observed replace control card, scan and report result of new card _____

                2. Do not proceed unless control card gives acceptable result, seek technical assistance _____

        3. 3M Petrifilm Plate Reader _____

            a. Store System Verification Cards in a clean, dry and enclosed container _____

            b. Scan and record System Verification Card result prior to the start of and at the end of each operation period _____

                1. Use Log File feature to automatically save electronic pass/fail result _____

            c. Scan and record System Verification Card result hourly with continuous operation _____

                1. Use Log File feature to automatically save electronic pass/fail result _____

            d. System Verification Cards used to check the function of the Petrifilm Plate Reader prior to reading test films (red, green and blue top lights, and backlights flash) _____

1. If inserting the System Verification Card results in an error message, remove and re-insert card _____

2. If an error occurs a second time, inspect card for visible dirt or defects, clean and re-insert card _____

3. If card gives a third error replace card, scan and report result of new card _____

4. Do not proceed unless System Verification Card gives an acceptable result, seek technical assistance _____

4. Advanced Instruments PetriScan Reader _____

a. Inspect scanner glass for spots and if necessary clean using a soft, lint-free cloth with a mild glass cleaner _____

b. Place templates 1 and 2, and two films with no growth in the PetriScan grid and scan _____

c. Count and record all results prior to the start of and at the end of each operation period _____

d. Scan, count and record template and no growth film results hourly with continuous operation _____

e. Template 1 gives count between 27 and 33 _____

f. Template 2 gives count between 190 and 210 _____

g. No growth films give a count of zero _____

h. If any results out of range _____

1. Inspect templates and films for defects and scanner glass for spots _____

2. If defect(s) found replace template or film and scan, count and record new result(s) _____

3. Do not proceed until template and no growth films give acceptable results, seek technical assistance _____

5. Maintain records _____

c. Examine each test film visually prior to placing into the reader _____

1. For films with no growth, assure reader count is zero _____

2. For atypical films, spreader, confluent growth, excessive growth around edge of film, etc., do not count with reader, record as appropriate using items 17 & 48 _____

48. Petrifilm Count _____

a. Count all colonies stained various shades of red, even those outside the circular indentation left by the spreader _____

b. See item 17 _____

c. Select spreader free films with 25-250 colonies and count all red colonies _____

d. If films from all dilutions yield < 25 colonies each, record actual number in lowest dilution _____

e. If all films from a sample show no colonies, record count as 0 _____

f. If films from all dilutions exceed 250 colonies, estimate (as per manufacturer specification)

49. Personal Errors _____

a. See item 19, or _____

b. If using an approved film reader (item 47b) analysts must perform monthly visual counts comparing to reader results (reader = one analyst) _____

1. If one analyst, count must be Ò 10% between visual and the reader result _____

2. If two or more analysts, use RpSm method (see current SMEDP) using the reader result as an analyst count _____

REPORTS

50. Reporting Counts _____

a. See item 20 _____

b. If the count is between 25 and 250, report count as Petrifilm Aerobic count/mL (PAC/mL) _____

c. If count is 0 to 24, report as < 25x reciprocal of the dilution as Estimated PAC/mL (EPAC/mL) _____

d. If count is > 250, report as EPAC/mL _____

e. When colonies exceed 100/sq cm, compute count by multiplying 100 x dilution factor x 20 sq cm and report as > computed count estimated _____

PETRIFILM COLIFORM COUNT METHOD

APPARATUS

51. Petrifilm Coliform Count (PCC) Films _____

52. Plastic Spreader _____

    a. Provided with Petrifilm films, smooth, flat side used _____

PROCEDURE

53. Selecting Dilutions _____

    a. For milk samples, 1 mL direct and/or decimal dilutions _____

    b. For other milk products use 1/10 dilution, must plate 10 mL, i.e., use 10 PCC films or see 64d _____

54. Identifying Films (as item 4) _____

55. Sample Agitation (as item 5) _____

56. Sample Measurement (as item 6 & 7) _____

57. Procedure _____

    a. Place films on level surface _____

    b. Lift top film and deposit 1 mL of sample above the center of the base film, touching off the last drop _____

    c. Carefully **roll** the top film into the inoculum, avoid trapping air bubbles _____

    d. Place the plastic spreader with the flat side down (item 52) on the top film and press down gently on the center of the spreader to distribute inoculum over growth area _____

    e. Leave films undisturbed for 1 min for gel solidification _____

    f. Incubate films within 10 min of solidification _____

INCUBATION

58. Incubation _____

    a. Place films in horizontal position, clear side up _____

    b. Stack films no more than 20 high _____

    c. Incubate 24±2 hr at 32±1C _____

COUNTING COLONIES

59. Counting Aids (see item 16) _____

60. Petrifilm Count _____

    a. Count only red colonies having 1 or more gas bubbles within 1 colony diameter _____

    b. Colonies with gas bubbles are confirmed, no other testing is required _____

REPORTS

61. Reporting Counts _____

    a. See item 20 _____

    b. If the count is between 1 and 154, report count as Petrifilm Coliform count/mL (PCC/mL) _____

    c. If count is 0, report as < 1 Estimated PCC/mL (EPCC/mL) _____

    d. If count is > 154, report as > 150 EPCC/mL _____

PETRIFILM HIGH-SENSITIVITY COLIFORM COUNT METHOD

APPARATUS

62. Petrifilm High-Sensitivity Coliform Count (HSCC) Films _____

63. Plastic Spreader for HSCC Films _____

PROCEDURE

64. Selecting Dilutions _____

    a. For milk samples, apply 5 mL direct and/or make decimal dilutions _____

    b. 1:5 minimum dilution required for: chocolate milk, cottage cheese, dip, evaporated milk, frozen yogurt, heavy and light cream, ice cream, sour cream, sweetened condensed milk and/or decimal dilutions _____

    c. 1:10 minimum dilution required for: butter, buttermilk, cheese, dry dairy products, yogurt and/or decimal dilutions _____

    d. 1:10 dilutions of milk or milk products test 10 mL (5 mL on two films) _____

65. Identifying Films (as item 4) _____

66. Sample Agitation (as item 5) _____

67. Sample Measurement (as item 6 & 7) _____

68. Procedure _____

a. Place film on level surface _____

b. Lift top film and deposit 5 mL of sample or dilution just above the center of the bottom film, touching off the last drop _____

c. Carefully **roll** the top film onto the sample gently to prevent pushing the inoculum off the film and to avoid trapping air bubbles _____

d. Place the plastic spreader (item 63) on the top film over the inoculum

e. Distribute sample with a gentle downward pressure on the handle of the spreader to distribute inoculum to the circular ridge of the spreader _____

f. Leave film undisturbed for 2-5 min for gel to solidify _____

g. Incubate within 10 min of solidification _____

INCUBATION

69. Incubation (see item 58) _____

a. Stack films no more than 10 high _____

COUNTING COLONIES

70. Counting Aids (see item 16) _____

71. Petrifilm Count (see items 18 & 60) _____

REPORTS

72. Reporting Counts _____

a. See items 20 and 61 _____

b. On 5 mL direct films report:

1. 1 to 4 colonies as < 1 coliform/mL or gm _____

2. 5 colonies as 1 coliform/mL or gm _____

3. > 5 colonies as 1 coliform for every 5 colonies counted, rounding up to the next number if not even multiples of 5 (ex. 11=3 coliforms/mL or gm) _____

c. 5 mL of 1:5 dilution provides a 1:1 sensitivity _____

d. 5 mL of 1:10 dilution provides a sensitivity of 2 coliforms/mL or gm, run 1:10 dilutions in duplicate to get a sensitivity of 1 coliform/mL or gm as required by the PMO _____